INVESTIGATIONS ON VITAMIN B_T

BY

KATHARINE ROMMELL WEIDMAN

A.B., Cornell University, 1948
M.S., University of Illinois, 1949

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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This investigation was carried out in collaboration with Dr. P. K. Bhattacharyya.

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INVESTIGATIONS ON VITAMIN B_T

Introduction

A growth factor required by the yellow mealworm, *Tenebrio molitor*, was first detected by Professor G. S. Fraenkel, Department of Entomology, University of Illinois, who named the substance Vitamin B_T. (1,2) Many animal tissues, yeast and milk, seem to contain considerable amounts of this factor, but few plant products show any B_T activity in the *Tenebrio* assays. (3,4) It was the purpose of this investigation, in cooperation with Dr. Fraenkel, to isolate Vitamin B_T in the pure form and study its physical and chemical properties.

Discussion Of Results

Second phenol extracts with Vitamin B_T activity of 3-6γ[1] were prepared according to the method of Fraenkel (5) from Borden's condensed whey solids (250-500γ) and Wilson's liver extract (100γ). The vitamin recovery from the whey to second phenol extract was about 20 per cent; recovery from the liver extract was roughly 30 per cent.

Chromatography of solids from second phenol extracts using alkaline alumina columns developed with 80 per cent aqueous methanol gave gummy fractions with B_T activities of 0.75γ. The vitamin was successfully crystallized from these fractions in one experiment. Two associated impurities with no B_T activity were separated from the vitamin on the column. Compound A melted at 256-258° (transition point 146-147°)

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1 The Vitamin B_T activity is expressed as the dry weight in micrograms (γ) of a preparation which must be added per gram of purified diet in the *Tenebrio* assay to give good survival and growth of the larvae as defined by the positive and negative controls.

and gave the correct analytical data for $C_4H_9N_3O_2$. Compound B melted at 257-258° and gave the following analytical data: C 41.93, H 6.15, N 36.91.

A 53-plate Craig countercurrent distribution of second phenol extract solids between phenol and water at pH 1.5 gave poor purification of the vitamin because of the large number of compounds present in the extract. A similar distribution of material active at 0.75γ, obtained by alkaline alumina chromatography of second phenol extract, gave efficient purification of B_T, as indicated by the close similarity between the experimental and theoretical distribution curves. The greatest weight of the vitamin was found in tube 20 or 21. An unidentified compound present in large amounts exhibited a weight peak at tube 9.

The hydrochloride of Vitamin B_T (M.p. 137-139°) was obtained from the active Craig fractions. The free vitamin (M.p. 196-198°) was obtained by converting the hydrochloride to the chloride-free form with IRA-400 and crystallization of the latter from anhydrous ethanol-acetone. Both the free vitamin and its hydrochloride were active at 0.37γ.

From the elemental analysis of the crystalline vitamin and an ebullioscopic molecular weight determination, the most probable formula for B_T appeared to be $C_7H_{15}NO_3$. The ultraviolet absorption spectrum showed only very weak end absorption around 200 mu.

The behavior of Vitamin B_T toward adsorbents, its distribution between phenol and water at different pH, and acid-base titration curve indicated its weakly basic nature. Because B_T failed to respond to any amine tests, and was acid-stable, alkali-labile, it was thought to be a disubstituted amide.

Hydrolysis of the purified vitamin with 2 N sodium hydroxide produced an amine and an acid. The amine gave a positive Simon test indicating that it was a secondary amine. It was identified as methylethylamine on the basis of the melting-points of its oxalate and picrate and elemental analy-

sis of the picrate. Studies are being continued on the structure of the acid portion of the molecule ($C_4H_8O_4$?).

Summary

A new growth factor, Vitamin B_T, has been obtained in purified crystalline form from whey and liver extract. Isolation steps included preparation of second phenol extracts, chromatography with alkaline alumina, and Craig countercurrent distribution between phenol and water. Vitamin B_T is an amide having the formula $C_7H_{15}NO_3$. Alkaline hydrolysis of the crystalline vitamin gave an unidentified acid and an amine which was characterized tentatively as methylethylamine.

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VITA

The author was born on October 18, 1927 in Camden, New Jersey. She attended the public schools in Woodbury, New Jersey, graduating from Woodbury High School in June, 1944. She attended Cornell University from September, 1944 until June, 1948 when she received her Bachelor of Arts degree. At Cornell she held Undergraduate and Ida H. Hyde Scholarships. In September, 1948 she entered the Graduate College of the University of Illinois, where she received the Master of Science degree in June, 1949. She held a University of Illinois Graduate Fellowship during the three academic years from September, 1948 to June, 1951. During the summer of 1950 she was recipient of a Borden research grant.

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